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Saline Flush During Excimer Laser Angioplasty: Short and Long Term Effects in the Rabbit Femoral Artery

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Background and Objective: **In this study, the effect of flushing saline on arterial wall damage (medial ruptures and necrosis), intimal hyperplasia, and arterial remodeling was determined. During excimer laser coronary angioplasty saline is flushed to reduce the size of explosive water vapor bubbles formed by intraluminal delivery of excimer laser pulses in blood.**

Methods: **In the femoral artery of the rabbit, 600 excimer laser pulses (308 nm, 50 mJ/mm^2 per pulse, 20 Hz) were delivered coaxially over a length of 20 mm in 10 bursts of 3 seconds each. In 24/48 procedures, saline was flushed (0.2 ml/s) via the guidewire channel. After 2 and 56 days, microscopic and angiographic results were compared.**

Results: **At 2 days, as compared to lasing in blood, saline flush had drastically reduced the incidence of dissections (2/12 vs. 11/12, $P < 0.002$), but had increased the extent of medial and adventitial necrosis. The latter is attributed to direct irradiation of the arterial wall. After 56 days, in the saline group, in the middle-distal part of treated segments, medial necrosis without intimal hyperplasia was observed. However, at the edges of these lesions, intimal hyperplasia and arterial shrinkage reduced the lumen.**

Conclusion: **Flushing saline during coaxial excimer laser pulse delivery significantly reduced the incidence of vessel wall ruptures, and prevented intimal hyperplasia formation in part of the lesion. The histologic findings at 56 days are attributed to the optical window which the saline flush provides for direct ultraviolet light irradiation of the arterial wall. Lasers Surg. Med. 23:128–140, 1998.** **© 1998 Wiley-Liss, Inc.**

Key words: laser angioplasty; dissections; intimal hyperplasia inhibition

INTRODUCTION

Laser angioplasty is an intervention technique based on removal of plaque by laser light which is delivered via flexible fiberoptic catheters in the stenosed artery. Due to its potential property of tissue ablation with minimal adjacent tissue damage [1], the development of coronary laser angioplasty has focused on the use of the XeCl excimer laser [2]. Improved multifiber catheter design with reduced dead space and larger opti-

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cally active area resulted in a high (90%) clinical success rate of excimer laser coronary angioplasty [3]. However, in approximately 13–45% of the procedures, dissections are observed angiographically [3–5]. These dissections, which reflect only a part of the mechanically induced collateral tissue damage observed by angioscopy [6], by intracoronary ultrasound imaging [7], or in histologic atherectomy specimens [8], might influence the initial results [4].

In contrast to earlier observations, in vitro [9–12] and in vivo [13,14] studies have demonstrated the explosive nature of tissue ablation by excimer laser pulses: Each ablative laser pulse absorbed by tissue or by blood will induce a rapidly expanding and imploding water vapor bubble (lifetime up to 300 μs, diameter up to 3 mm) and stress waves. We demonstrated that within tissue, the explosive water vapor bubble expansion separates tissue layers [13]. Intraluminal bubble expansion and collapse in blood induced a microsecond dilation and subsequent invagination of the femoral and iliac artery of the rabbit, which resulted in rupture and abrasion of the internal elastic lamina and in medial dissections [14]. It was hypothesized that the rapidly expanding and imploding vapor bubble contribute to dissections of the arterial wall by two potential mechanisms: 1) by expanding into adjacent tissue seeking the line of least resistance [13,15,16] and 2) by microsecond dilation and invagination of the adjacent arterial wall [14]. Decreasing the size of the bubble that is induced in blood and in tissue may reduce the extent and incidence of dissections.

During laser angioplasty, the laser light will be absorbed by the target tissue as well as by blood. A new strategy to reduce the size of a bubble formed in blood during excimer laser angioplasty applies continuous flush of saline solution during lasing. Blood or contrast medium with relatively high absorption coefficients for UV light are replaced or at least diluted by saline with a much lower absorption coefficient [12,17,18]. This simple strategy decreases the volume of the excimer laser induced bubble and pressure waves in blood only (and not in tissue) [19]. Preliminary experimental [17,20] and clinical [17,21] studies demonstrate that the incidence of significant dissections is drastically reduced by providing a saline flush during the laser pulse. The trade off, however, may be that diluting the highly absorbing blood, with a penetration depth at 308 nm of approximately 30 μm [14], with the almost transparent saline provides an optical window that allows direct irradiation of the arterial wall by the UV light. The effect of flushing saline during excimer laser light delivery on arterial wall damage and its repair mechanism has not been documented yet.

The aim of this study was to assess the effect of flushing saline during intraluminal excimer laser pulse delivery on arterial wall damage, intimal hyperplasia, and arterial remodeling [22,23]. We therefore compared angiographic and histologic results at 2 and 56 days after intraluminal delivery of 600 excimer laser pulses over a segment length of 20 mm in the rabbit femoral artery with or without saline flush.

MATERIALS AND METHODS

Excimer Laser Angioplasty

Twenty-four healthy ELCO rabbits weighing 4.5–5.5 kg were used in this study. Animal care conformed to the positions of the "American Heart Association on Research Animal Use" and to the Faculty Commission on Animal Experiments of the Utrecht University. The rabbits were housed in groups and received a normal diet throughout the course of the experiments.

Before each procedure, the rabbit was anaesthetized as described previously[14] and a 5 F sheath (Cordis, Europa NV, Roden, The Netherlands) was inserted into the right carotid artery with its tip in the descending aorta. Rabbits received a dose of 100 IU Heparin/kg. Angiography of the femoral and iliac arteries was performed by a C-arm (Philips BV 26, Philips, Eindhoven, The Netherlands, telebrix 350, Guerbet, Roissy, France) before and after the procedure and at follow-up. Angiograms were digitized and stored.

The experiments were performed with a XeCl excimer laser (Technolas MAX-10, Germany, wavelength 308 nm, pulse length 115 ns) at a pulse frequency of 20 Hz. A 4.5 F coronary multifiber catheter (diameter 1.5 mm) consisting of approximately 160 fibers of 50 μm diameter each, which delivered approximately 16 mJ per pulse (radiant exposure of 50 mJ/mm^2 per pulse), was used. The laser catheter was advanced under fluoroscopy into the femoral artery. In 10 bursts of 3 seconds each, a total of approximately 600 excimer laser pulses (20 Hz) were delivered via the multifiber catheter. Bursts were separated by intervals of 2 seconds, which was controlled by an external switch box. During laser light delivery, the catheter was pulled back under fluoroscopy (0.67 mm/s) over a length of 20 mm. The images of

the starting and end point of the multifiber catheter were digitized and stored. In each animal, in one artery, the laser pulses were delivered in blood (n = 24), whereas in the contralateral artery, a saline flush (0.2 ml/s, 12 ml total) was simultaneously provided via the guidewire channel. Saline flush started 10 seconds prior to laser light delivery and was maintained in the 2 seconds interval between pulse trains. Laser light delivery in blood or saline was randomized over the left and right femoral artery, as well as over the first and second treated femoral artery.

After all procedures, the catheter and sheath were withdrawn and the carotid artery was ligated. The wound was closed with vicryl 4-0. The output energy of the catheter and the number of delivered laser pulses were noted.

To assess the cumulative UV light dose (in mJ/mm^2) which the arterial wall was exposed to during the laser angioplasty procedure in the flush group, the laser light intensity profile was measured in vitro. Therefore, 650 nm continuous wave laser light was delivered via the XeCl excimer laser incoupling system into the multifiber catheter. In front of the catheter the intensity of the red laser light was measured in water by means of a 200 μm diameter spherical diffusing tip connected to a 100 μm diameter fiber. In this way, the radiant exposure of one excimer laser pulse could be estimated. For the average number of delivered excimer laser pulses and for the average length of the pull back maneuver of the multifiber catheter, the cumulative radiant exposure at a certain radial distance from the catheter could be estimated.

Sacrifice and Histologic Processing

Two and 56 days after excimer laser angioplasty, the rabbits were sacrificed by an overdose of sodium pentobarbital (60 mg/ml i.v.). A midabdominal incision was made and the descending aorta and inferior vena cava were isolated and ligated cranially. The femoral arteries were saline perfused in situ at 60 mm Hg.

For the 2 days survival group, the patent femoral arteries were cut and rolled longitudinally (*Swiss rolls*) and fixed for more than 24 hours, using formalin 4%. For the 56 days survival group and the occluded segments of the 2 days survival group, the saline perfused arteries were pressure perfused by a mixture of contrast medium (Telebrix 350) and Agar at a temperature of 50°C. This mixture, which congealed in the arteries, prevented collapse of the arteries during fixation. The arteries were then fixed in situ and periadventitially, using formalin 4%. After more than 24 hours of fixation, the femoral arteries were divided in 6 mm segments.

All segments (2 and 56 days survival) were dehydrated and embedded in paraffin. The *Swiss rolls* and the cross sections were cut in duplicate 5 micron sections at intervals varying from 100 to 200 micron and stained with Hematoxylin and Eosin and with Elastin van Gieson. In the 56 days survival groups, endothelial cells were identified with a monoclonal antihuman antibody JC70, (1:50 μl, Dakopatts), which recognizes a membrane bound glycoprotein identical to the CD31 group of epitopes. Endothelial cell coverage was scored by the presence of anti-CD31 positive endothelial cells as percentage of lumen circumference. Proliferation was detected with the monoclonal MIB-1 which reacted to the human nuclear antigen Ki-67 (1:50 μl, Immunotech). Horse biotinylated antimouse (1:200 μl, Vector) was used as a secondary antibody. Immunohistochemistry was performed as described previously [24]. Occluded cross sections were stained with Martius Scarlet Blue to identify fibrin.

Angiographical Evaluation

The angiographic diameters of the femoral arteries were measured using a semiautomated program with digital calipers. The quantitative edge detection algorithm is applied on the digitized gray value of a proposed line perpendicular to the center axis of the lumen. The gray value distribution along the perpendicular line has its maximum outside the lumen and its minimum in the middle of the lumen. The edge of the lumen was defined by the pixel with a gray value equal to the average of the maximum and minimum. The diameter of the artery was calculated by this full-width-half-maximum distance. In each artery, lumen diameters were measured at six positions: one proximal and one distal reference site, and four sites equidistantly spaced (6 mm) within the treated segment. To use equal positions at different time points (pre and post procedure and at follow up), the six positions were documented relative to an anatomic landmark. Angiography was calibrated using a radiopaque ruler.

The mean and minimal luminal diameter of the lesion (the irradiated arterial segment) were determined. Acute gain was defined as the difference between post and pre procedure mean lumen diameters. Late loss was defined as the difference between follow-up and post procedure mean lumen diameters.

TABLE 1. Arterial Wall Damage at 2 Days Follow-up: Incidence of Dissections and Occlusions as Well as the Segment Length With Medial and Adventitial Necrosis

	n[a]	No. Pulses[b]	Irradiated Length [mm]	Incidence of		Segment Length [mm]	
				Occl.[c]	Diss.[d]	Med. Necr.[e]	Adv. Necr.[f]
Blood	12	592 ± 14	20.6 ± 2.6	2	11 *	10.7 ± 4.1 *	6.9 ± 5.0 *
Saline	12	589 ± 19	22.5 ± 3.4	0	2	16.0 ± 3.8	14.1 ± 4.2

[a]n: number of arteries.
[b]No. pulses: number of delivered laser pulses per lesion.
[c]Occl.: occlusion.
[d]Diss.: medial dissections or internal elastic lamina ruptures.
[e]Med. Necr.: medial necrosis.
[f]Adv. Necr.: adventitial necrosis. Data are presented as mean ± Standard Deviation.
*P <0.05

Microscopic Evaluation

Arterial wall damage (dissections and necrosis) was assessed in the 2 days survival group in the Elastin van Gieson and Hematoxylin and Eosin stained sections. Dissections identified by red blood cells in the media or by abrasion of the internal elastic lamina, as well as medial and adventitial necrosis, as indicated by the absence of nuclei, were evaluated qualitatively and quantitatively and expressed as incidence, number of sites per lesion and segment length in mm.

In the 56 days survival group, lumen, intima, and media bounded area were assessed quantitatively in mm^2 in Elastin van Gieson stained cross sections (Analyze, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN). The analyzed cross sections were harvested from six locations similar to the angiographic measuring positions: one proximal and one distal reference, and four cross sections in the irradiated part of the artery. From these areas, in each cross section, the (mean) lumen diameter, intimal hyperplasia thickness, and media bounded diameter were calculated assuming circular anatomy [22]. Proliferation was scored by the number of MIB1 positive nuclei per unit of length of the circumference of the lumen [/mm].

Luminal narrowing after interventional injury is the result of both intimal hyperplasia formation and remodeling of the artery [22,23,25]. Remodeling of each lesion was defined as the difference between mean lumen narrowing on serial angiography (late loss) and two times the average thickness of the intima in histology at follow-up, as described before [22].

Statistical Analysis

Angiographic and histologic data are presented as mean ± standard deviation (SD) or as mean ± standard error of the mean (SEM). Differences in angiographic lumen diameters and intimal hyperplasia thickness between the saline flush and blood group were assessed by a two factor repeated analysis of variance (SPSS for Windows version 6.1, SPSS Inc., Chicago, Illinois). The incidence of various types of damage was evaluated by a χ^2 test. Continuous variables were compared using paired and unpaired t-tests.

Differences were considered significant at a level of $P < 0.05$. A sample size of 12 arteries would result in 80% power to detect a 30% decrease in the incidence of dissections in the saline flush group, compared to an expected 90% incidence of dissections in the blood group [14].

RESULTS

2 Days Survival

Angiography. The length of the treated arterial segment was similar in both groups, as well as the number of laser pulses (Table 1). The laser energy measured before and after the procedure was 16.3 ± 0.5 and 13.9 ± 2.2 mJ per pulse ($P = 0.001$), respectively. The mean angiographic luminal diameters at the lesion before, directly after, and at follow-up 2 days after laser light delivery are presented in Fig. 1. Between the saline flush and blood group, no statistically significant difference in luminal diameters was observed. Within these groups, the angiographic diameter of the irradiated segment after the procedure was smaller than the diameter measured before the procedure, indicating a negative acute gain, and at follow-up ($P < 0.05$).

Arterial wall damage. In the blood group, a damage pattern was observed similar to our previous study [14]: Focal areas (3 ± 2 per lesion)

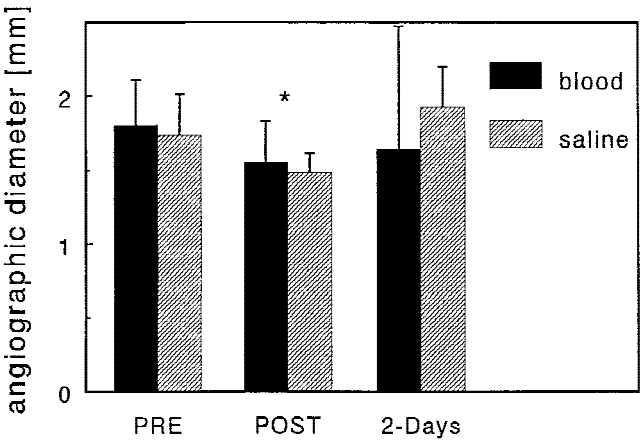


Fig. l. Average angiographic diameters (± SD) of normal rabbit femoral artery before (Pre), directly after (Post) and at 2 days (2-Days) after intraluminal delivery of 600 excimer laser pulses at 50 mJ/mm^2 in a blood or in a saline environment. *: $P < 0.05$ compared to pre and follow-up mean diameters.

with medial dissections stained by red blood cells or internal elastic lamina ruptures were accompanied by medial necrosis with infiltrated leukocytes, and occasionally by adventitial necrosis (Fig. 2A). Two arteries were occluded at 2 days follow-up.

Saline flush drastically reduced the incidence of medial dissections or internal elastic lamina ruptures (2/12 vs. 11/12 arteries, Table 1, $P < 0.002$). Also, in the saline flush group, medial and adventitial necrosis was not focally distributed as in the blood group, but was found over a considerable length of the treated artery (Fig. 2B). Medial necrosis was almost complete (Fig. 2B) and in 10/12 arteries distributed over one large segment. At the edges of the irradiated segments, a gradual decrease in depth of medial necrosis was observed (Fig. 2B). The average necrotic segment length was larger in the flush group. Necrotic segments were rarely infiltrated by leukocytes. Neither medial ablation nor mural thrombus formation in the arterial segments was observed at follow-up.

56 Days Survival

Angiography. The angiographic diameter and length of the treated artery were similar in the blood and saline groups, as well as the number of laser pulses (Table 2). The laser energy measured before and after the procedure was 16.3 ± 0.7 and 14.7 ± 1.0 mJ per pulse, respectively ($P < 0.0005$). The angiographic diameter changes of the lesions at the different time points are depicted in Fig. 3. After 56 days of survival 11/24 arteries were occluded (Table 2). Before the procedure, the angiographic diameter of the occluded arteries was smaller compared to that of the patent arteries (1.61 ± 0.30 vs. 1.79 ± 0.21 mm, $P < 0.05$).

In the blood group, 7 out of 12 arteries were occluded. In four out of five patent arteries, the lesions were characterized by a 2 cm long stenosis, with a smooth lumen boundary. For all lesions, the average angiographic diameter was decreased compared to the pre and post procedure diameters, which resulted in a highly significant late loss ($P < 0.0005$ compared to zero).

In the saline flush group, four arteries were occluded. The angiographic lumen of the lesions in the eight patent arteries was more irregularly shaped than in the blood group. In six out of eight patent arteries, at least one of the four segments within the lesion revealed a larger lumen compared to the post procedure. Therefore, the average late loss in all arteries and in the patent arteries only was smaller compared to that of the blood group ($P < 0.04$). However, for all arteries as well as for the patent arteries only, the minimal and average angiographic diameter at follow-up were not statistically significant different between the saline flush and blood group.

Histochemistry. The angiographic data were confirmed by the histomorphometry. Occluded lesions were characterized by recanalized thrombi and intimal hyperplasia within a shrunken artery (histologic media bounded diameter 0.78 ± 0.36 mm). In patent arteries, a regular intimal hyperplasia (mean thickness 0.07 ± 0.05 mm), with smooth muscle cells oriented longitudinally near the internal elastic lamina and circumferentially near the lumen, was observed. In both the saline and blood group, internal elastic lamina ruptures were present in at least one segment of 8 out of 12 lesions. Furthermore, mural thrombi identified by fibrin were present in 4 out of 12 lesions of the saline group.

In the patent arteries of the blood group, intimal hyperplasia was present in the irradiated arterial segment (mean thickness 0.07 ± 0.05 mm, N = 5) and in its proximal reference segment (Fig. 4A and Fig. 5A). However, in the distal reference segment, no intimal hyperplasia was observed (Fig. 4B and Fig. 5A). The media and adventitia were restored. The luminal surface was almost completely reendothelialized and smooth muscle cell proliferation in the intima and media was low (Fig. 6A). No fresh thrombi were observed.

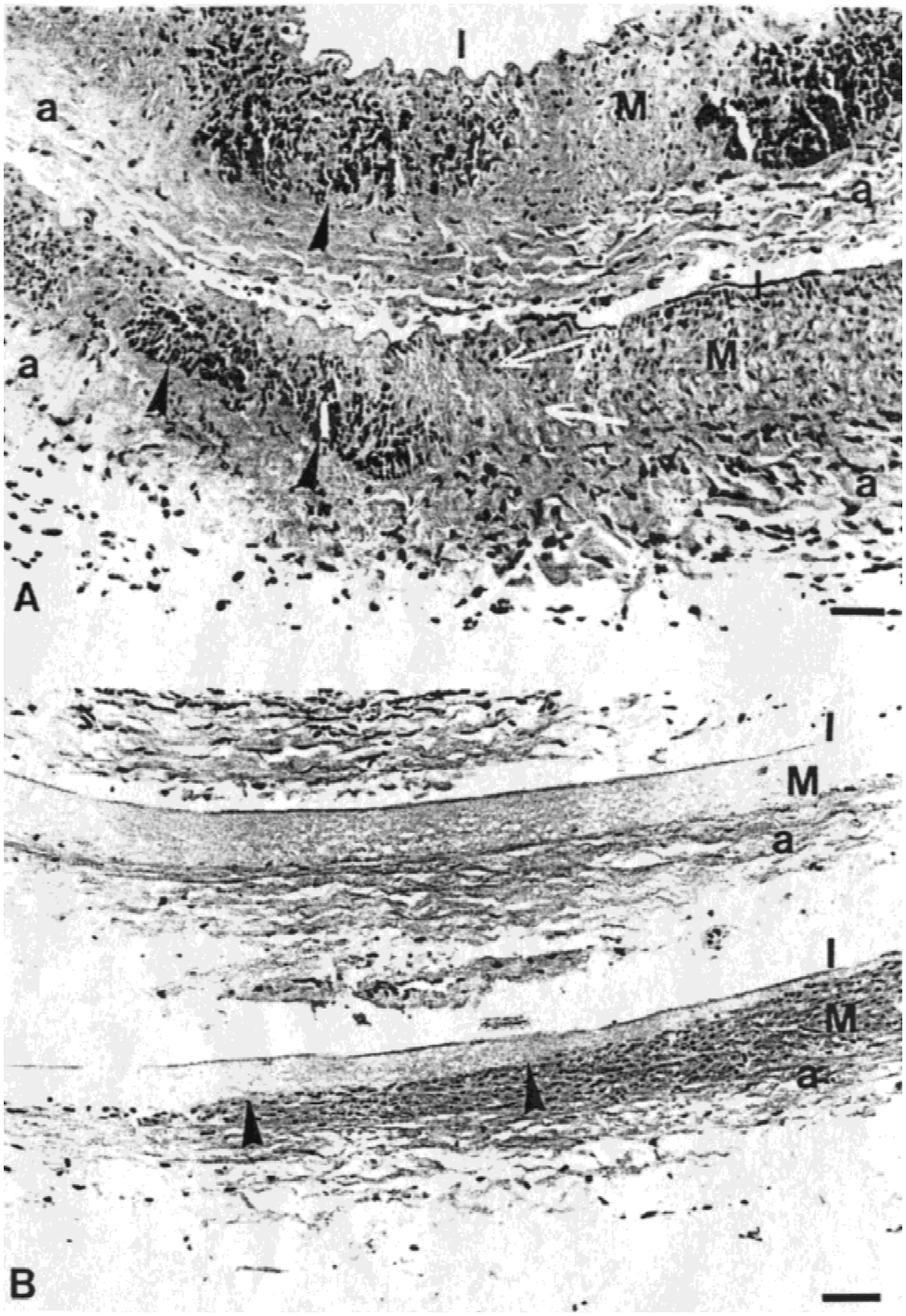


Fig. 2. Longitudinal section (*Swiss rolls*, Hematoxylin and Eosin stained) of normal rabbit femoral artery 2 days after intraluminal delivery of 600 excimer laser pulses at 50 mJ/mm^2 in a blood **(A)** or in a saline environment **(B)**. In both panels, the top part is the cranial segment of the femoral artery. Note in panel A the medial dissections filled with erythrocytes and focal necrosis (black arrows), and the abrupt change in medial necrosis (white arrows). In panel B, note the gradual change over the length of the artery in medial and adventitial necrosis at the (caudal) edge of the irradiated segment (arrow) and the almost total medial and adventitial necrosis with hardly any infiltration of leukocytes towards the center of the lesion. a = adventitia, I = internal elastic lamina, M = media, Scale bar = 0.05 mm.

TABLE 2. Number of Laser Pulses, Length of the Irradiated Arterial Segment, Angiographic Diameter Before Procedure, and Incidence of Occlusions at Follow-up at 56 Days After Excimer Laser Light Delivery in Blood or in Saline

	n[a]	No. pulses[b]	Irradiated Length [mm]	Pre-Angiographic Diameter [mm]	Incidence of Occlusions
Blood	12	592 ± 4	20.3 ± 2.1	1.73 ± 0.34	7
Saline	12	594 ± 5	21.6 ± 1.2	1.68 ± 0.18	4

[a]n: number of arteries.
[b]No. pulses: number of delivered laser pulses per lesion. Data are presented as mean ± Standard Deviation.

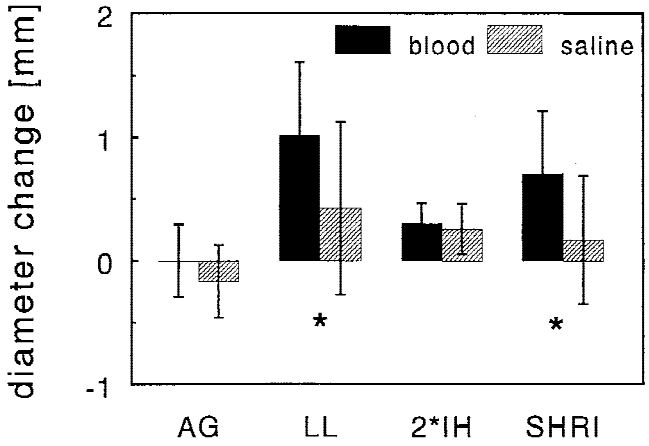


Fig. 3. Changes in angiographic diameters of normal rabbit femoral artery: Acute gain (AG) and late loss (LL) at 56 days and the contribution of intimal hyperplasia (2*IH) and arterial shrinkage (SHRI) to the late loss after intraluminal delivery of 600 excimer laser pulses at 50 mJ/mm^2 in a blood or in a saline environment (mean ± SD). *: $P < 0.05$ blood vs. saline.

In the patent arteries of the saline flush group, the intimal hyperplasia was not uniformly distributed over the irradiated segment (Fig. 5B). The mean thickness of the intimal hyperplasia over the entire lesion was 0.08 ± 0.06 mm (N = 8, $P = 0.89$ compared to the mean thickness in the blood group). In general, at the proximal edge of the lesions and in the proximal and distal reference segments, intimal hyperplasia was present, whereas, in the middle and distal part of the lesions, intimal hyperplasia was absent. This distribution of intimal hyperplasia differed between the saline and flush group ($P = 0.008$). The adventitia was repopulated. However, in the middle-distal part of the lesions, parts of the media were still acellular (Fig. 4C and Fig. 4D) with an only partially endothelialized luminal surface (Fig. 6B). In the acellular parts of the lesions, erythrocytes and eosinophil granulocytes were observed. The absence of endothelium correlated with increased cell proliferation in the intima and media (R = 0.4, $P < 0.0002$).

Remodeling. The angiographic loss in diameter could only partly be explained by the amount of intimal hyperplasia (Fig. 3), which indicates the importance of arterial wall shrinkage on luminal narrowing after arterial wall injury. The larger late loss in the blood group is explained by the larger arterial wall shrinkage compared to the saline flush group (0.71 ± 0.51 mm vs. 0.17 ± 0.52 mm, $P = 0.021$). In the occluded lesions, 70% of the late lumen loss (1.31 ± 0.51 mm) was due to arterial shrinkage.

UV light dose. The radiant exposure for one 15.5 mJ excimer laser pulse (Fig. 7A) was estimated for different radial distances (0.75, 0.84, and 1.00 mm) from the center of the 1.5 mm diameter multifiber catheter. These radial distances represent the edge of the catheter and the average and maximal measured angiographic arterial radius, respectively. For larger radial distances, due to the divergence of the laser light, the maximal radiant exposure decreases and its position shifts. Furthermore, note that the laser light can irradiate the arterial wall up to 5 cm in front of the catheter. The cumulative dose (for 589 pulses delivered over 21 mm) for the above mentioned radial distances are depicted in Fig. 7B. Note that the cumulative radiant exposure has its maximum a few mm in front of the distal starting point of laser angioplasty. Two times the measured intimal hyperplasia thickness is also depicted in Fig. 7B.

DISCUSSION

In this study, we evaluated the effect of flushing saline during excimer laser irradiation on arterial wall damage and its healing response. The main findings are that saline in front of the catheter tip reduced the mechanical damage in the form of medial dissections, but increased the medial and adventitial necrosis. Furthermore, up to 56 days after laser light delivery during saline flush, a distinct lesion was observed, with a partly

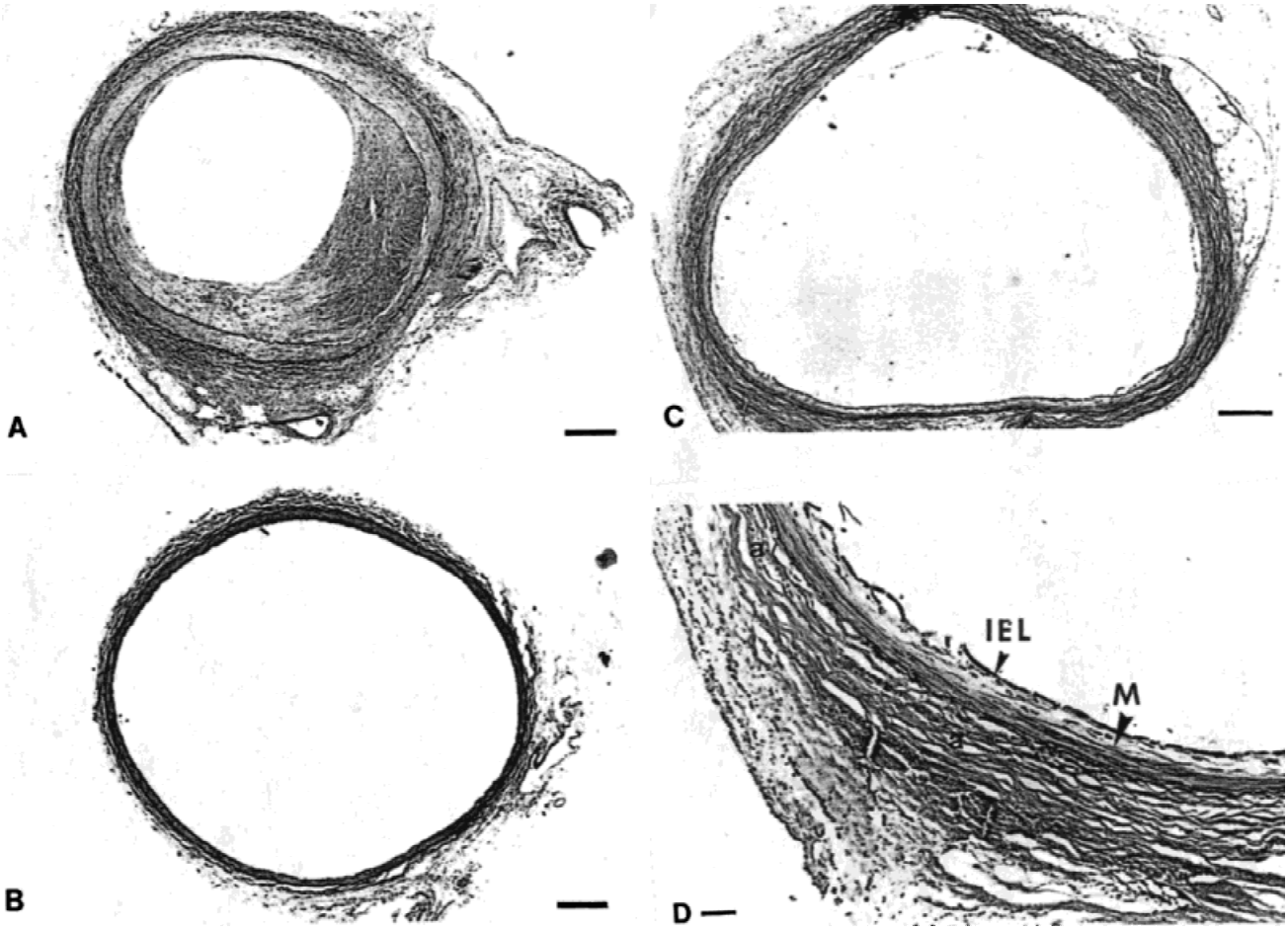


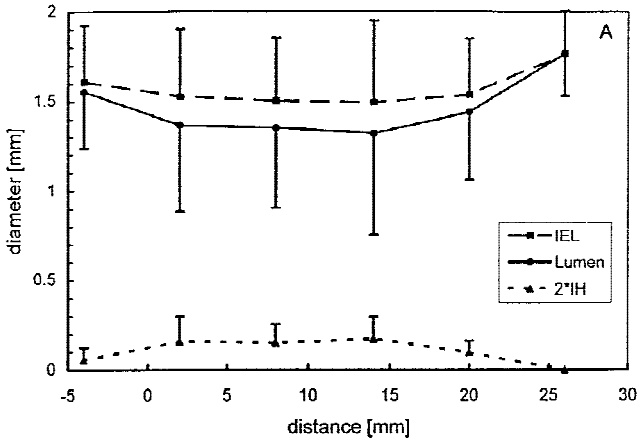
Fig. 4. Cross sections (Elastin van Gieson stained) of lesions induced 56 days after excimer laser pulse delivery: In blood (A and B, bar = 0.2 mm) and in saline (C, bar = 0.2 mm and D, bar = 0.05 mm). **A:** Cross section in the middle of the lesion. **B:** Cross section of its distal reference. Note in panel B the absence of intimal hyperplasia and in panel A the shrinkage of the artery compared to its reference. **C:** Cross section in the middle of the lesion. Note in the magnification, **D,** the absence of intimal hyperplasia and the platelet rich microthrombi formed on the luminal surface (C) and the partially failed restoration of the media (D). IEL: internal elastic lamina; M: media.

acellular media, and absence of both endothelium and intimal hyperplasia in the middle and distal part. In both the blood and saline flush group, luminal narrowing was caused by intimal hyperplasia as well as by local arterial shrinkage.

Damage Mechanism

Applying saline flush during excimer laser angioplasty reduces the absorption coefficient of the immersion fluid from 30 mm^{-1} to virtually zero mm^{-1}. This simple strategy decreases the volume of the excimer laser induced bubble in blood only (but not in tissue) [12,17,18] and prevents the augmented bubble formation by delivery of excimer laser pulses in contrast medium. However, in addition to smaller or even absent explosive bubbles [20], flushing saline will result in direct irradiation of the arterial wall by the diverging and scattered 308 nm excimer laser light pulses.

In the saline flush group, the necrotic part of the artery was distributed over one (10/12) or two (2/12) large segments, whereas in the blood group medial and adventitial necrosis was more focal and patchy (Fig. 2). In a preliminary in vivo study [20] we demonstrated, by means of an optical reflection measurement as described by Brinkmann et al. [26], that the applied flush was sufficient to prevent bubble formation in blood. Furthermore, flushing saline during excimer laser irradiation drastically reduced the incidence of medial dissections (Table 1, Fig. 2). In the flushed arteries, the cumulative radiant exposure, which was delivered in 50 seconds, was at maximum 80 mJ/mm^2



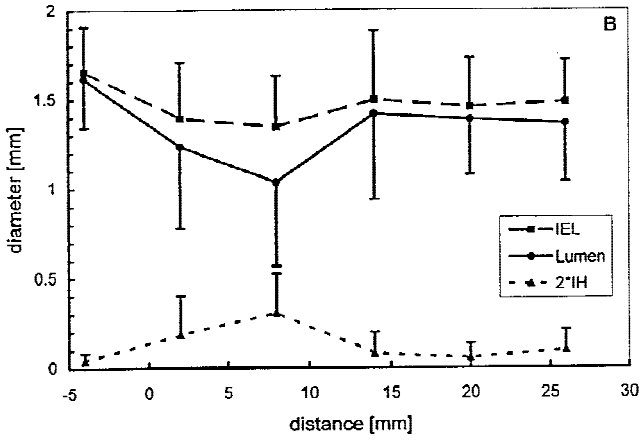
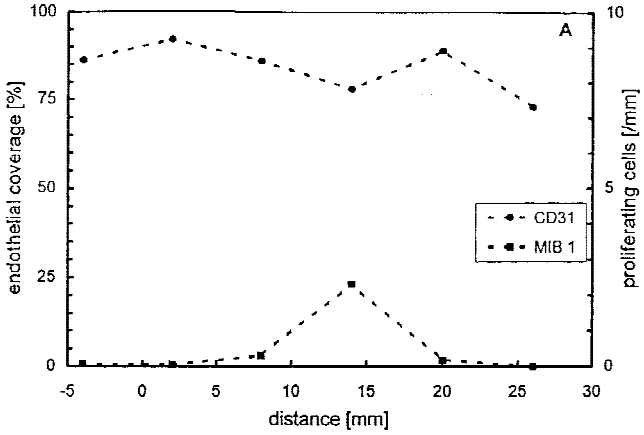


Fig. 5. The media-bounded diameter (solid square, striped line), lumen diameter (solid circle, solid line) and 2*intimal hyperplasia thickness (2*IH, solid triangle, dotted line) of excimer laser induced lesions in the blood group (**A,** N = 5) and in the saline flush group (**B,** N = 8) as a function of distance to its proximal end point. Note the large contribution of arterial shrinkage to the luminal narrowing. Data points are mean ± SD. The treated segment extended approximately from 21 mm (starting point) to 0 mm (end point). Intimal hyperplasia thickness distribution is different between the two groups ($P = 0.008$).



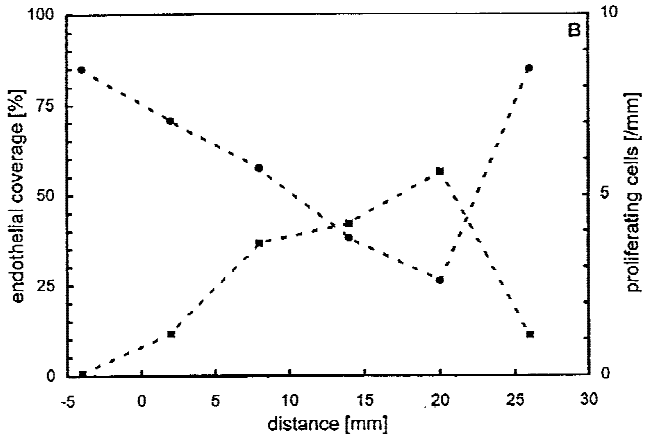


Fig. 6. The endothelial cell coverage as percentage of the circumference of the arterial wall (solid circle, left Y-axis) and the number of proliferating cells per mm circumference of the arterial wall (solid square, right Y-axis) 56 days after excimer laser induced injury. Only patent lesions in the blood group (**A,** N = 5) and in the saline flush group (**B,** N = 8) are displayed as a function of distance to its proximal end point. The treated segment was approximately between 21 mm (starting point) to 0 mm (end point).

(Fig. 7B). We hypothesize that an average laser light intensity of only 80/50 = 1.6 mW/mm^2 is insufficient to induce thermal necrosis. Towards the edges of the irradiated segment, the UV dose decreases to zero, which explains the observed gradual decrease of necrotic zone thickness in the distal part of the necrotic segment. Therefore, in the saline flush group, we attribute the medial and adventitial necrosis with hardly any infiltration of leukocytes to direct irradiation of the arterial wall (photochemical cell damage), whereas in the blood group, both dissections and necrosis are attributed to explosive intraluminal vapor bubble expansion and subsequent equally rapid implosion (mechanical damage).

Repair Mechanism

Both laser light delivery strategies (in blood or in saline) induced arterial stenoses and occlusions. The average and minimal angiographic diameters of the stenoses were not different between the two groups. Thus, flushing saline during excimer laser light delivery failed to improve the angiographic outcome. However, angiography showed differences in shape of the patent lumens between the two groups, which was confirmed by the histologic evaluation. In the blood group, the

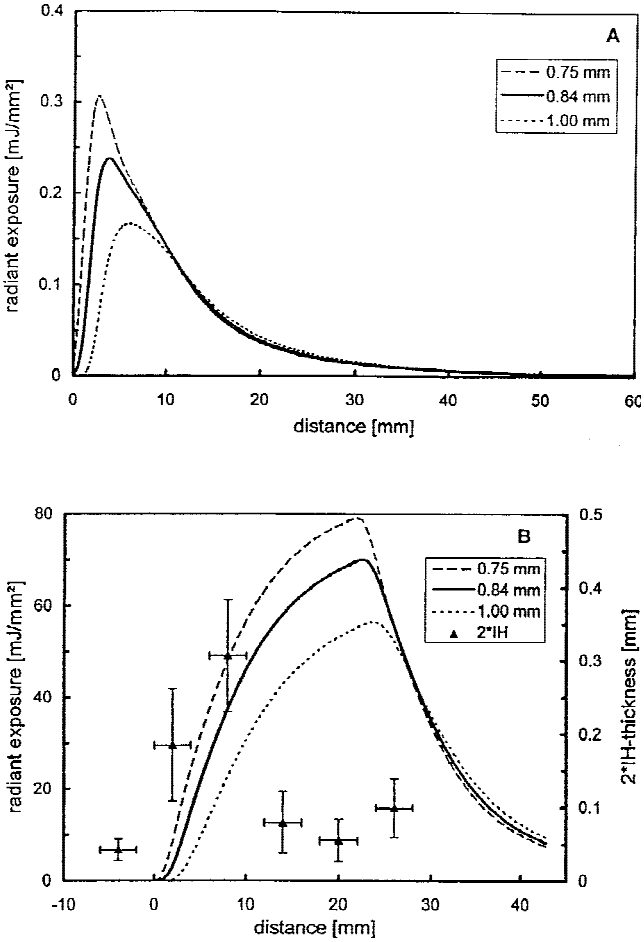


Fig. 7. Calculated radiant exposure as a function of distance from the catheter tip for different radial distances (0.75, 0.84, and 1.00 mm) from the center of the catheter tip. **A:** Radiant exposure due to one 15.5 mJ excimer laser pulse. **B:** Cumulative radiant exposure due to 594 laser pulses (as shown in A) equidistantly delivered over a distance of 21.6 mm. In Fig. B, mean intimal hyperplasia thickness ± SEM is also depicted. Horizontal error bars indicate possible mismatch between angiographic and histologic position.

lesion was distinct and with a uniform intimal hyperplasia in the lesion. The saline flush group had lesions with a horse saddle profile: Intimal hyperplasia and luminal narrowing was predominantly present at the edges of the lesions (Fig. 5). In the middle-distal part of the saline flush lesions, intimal hyperplasia was absent and some medial areas were devoid of smooth muscle cells and had no endothelial cell coverage. The distal reference segments in the blood group were without intimal hyperplasia, whereas in the saline flush group, intimal hyperplasia was found in 10/12 distal reference segments.

The intimal hyperplasia distribution in the saline flush group might be explained by a dose-response effect of nonablative UV light absorbed by the arterial wall. Compared to the proximal part of the lesion and to the reference segments, the middle-distal part of the irradiated segment was exposed to more UVB laser light (Fig. 7B). In this part of the artery, intimal hyperplasia thickness was smaller. The decreasing UV dose towards the edges of the irradiated segments may have been too low to prevent intimal hyperplasia formation. Note, however, the large variation in intimal hyperplasia thickness (vertical error bars) and the possible large variation in absorbed cumulative light dose. The latter variation is not only due to the unknown catheter alignment inside the artery ($0.75 < \text{radial distance} < 1.00$ mm), but also due to possible mismatch of angiographic and histological position (the horizontal error bars).

The intimal hyperplasia distribution might also be explained by the partly endothelialization of the intimal hyperplasia at the edges of the lesion (Fig. 6). This leading edge of regenerated endothelium next to the adjacent denuded arterial wall might promote more intimal hyperplasia formation [24,27]. In the flush group, the number of proliferating smooth muscle cells was still elevated, indicating a potentially progressive intimal hyperplasia formation. In contrast, in the blood group lesions, at 56 days cell proliferation rate was low, which is in accordance with a previous report [28].

The number of occlusions (11/24) was surprisingly high at 56 days compared to the 2/24 occlusions at 2 days. Furthermore, whereas at 2 days in only two lesions small internal elastic lamina ruptures were observed in the flush group, at 8 weeks, in 8 out of 12 lesions small parts of the internal elastic lamina were absent. Mural thrombi were found in 4 out of 12 arteries. The mechanism of this late response is not known. It is conceivable that elastin absorbs the ultraviolet excimer laser light, because the peak absorption occurs at 300–340 nm [29]. It might be possible that the 308 nm excimer laser light degenerated the internal elastic lamina, which then slowly looses its consistency and its barrier function. This also might explain the presence of red blood cells in the cell depleted parts of the media, 56 days after laser light delivery. More research has to be performed to address the effect of UVB on elastin and collagen in the arterial wall.

Intimal Hyperplasia and Remodeling

In both groups, intimal hyperplasia and arterial shrinkage contributed to the angiographically observed late loss in luminal diameter, a result which was also been reported after balloon dilation of rabbit arteries [22]. Furthermore, in the flush group, the focally prevented intimal hyperplasia formation due to direct irradiation by ultraviolet excimer laser was not compensated by arterial shrinkage. Consequently, arterial shrinkage was smaller than in the flush group, which resulted in a smaller late loss compared to that of the blood group (Fig. 3). In this combined angiographic and histological method, remodeling was assessed by the difference of average late loss and twice the average intimal hyperplasia thickness within each lesion. However, due to the negative acute gain, the real contribution of arterial shrinkage to luminal narrowing is probably underestimated. At 2 days, a small late gain was observed, indicating that remodeling is a late response after intervention and does not occur in the first 48 hours.

Limitations of the Study

Excimer laser angioplasty was regarded as an alternative approach to balloon angioplasty in the treatment of obstructive coronary lesions longer than 10 mm [30]. In practice, the multifiber catheter is gently pushed against the stenosis and during a 3 to 5 second long series of laser pulses the catheter is advanced over a guidewire through the lesion. In this study, the guidewire channel was used for saline perfusion and the mean catheter/artery diameter ratio was 0.88. Therefore, to reduce the risk of perforation, the catheter was pulled back during excimer laser light delivery.

The mechanism of luminal enlargement by excimer laser angioplasty is still unclear. Intravascular ultrasound studies by Honye et al. [31] and Mintz et al. [32] demonstrated that only a small part of the atheroma is ablated by excimer laser irradiation. The microsecond dilatory effect by the explosive bubble formation may substantially contribute to the recanalization mechanism [32,33]. Even in a saline field, ablative excimer laser pulses create rapidly expanding and imploding vapor bubbles, which induce small dissections in adjacent tissue [13,17]. In this study, atherosclerotic tissue in front of the catheter was absent. However, during excimer laser angioplasty, the multifiber catheter may also be in close contact to and directly irradiate the relatively normal arterial wall in eccentric lesions and in shrunken arteries [25]. Thus, whereas during saline flush, bubble formation was absent in this study, in obstructed arteries it will be present anyhow. Deckelbaum et al [21]. demonstrated that flushing saline did reduce the severity but not the number of angiographically observed dissections.

Clinical Implications

This study demonstrated that a simple flush and bath technique reduced the arterial wall damage induced by rapidly expanding and imploding vapor bubbles in blood. Displacement of the highly absorbing blood by the transparent saline allows direct irradiation of the arterial wall by the ultraviolet laser light, which will result in increased medial and adventitial necrosis. The in vitro experiments demonstrated that the laser light can irradiate the arterial wall up to 5 cm in front of the catheter. In fact, in a pilot experiment in which the iliac and femoral artery of a rabbit was exposed as described previously [14], we observed laser induced fluorescence up to centimeters in front of the catheter tip, when the lumen was flushed with saline during excimer laser irradiation. Furthermore, in the flushed arteries, the cumulative radiant exposure at the arterial wall is not constant, but is maximal at the distal part of the lesion, which may explain the inhibition of intimal hyperplasia in that part of the lesion. However, due to bending of the coronary arteries, the extent and distribution of the UVB laser light illumination at the arterial wall will be different during ELCA. For treatment of in stent restenosis by laser angioplasty [34], the curvature of the vessel segment might be less, and it will be interesting if a similar arterial response to UVB laser light will be found at follow-up.

CONCLUSIONS

From this in vivo study in the rabbit, we conclude that flushing saline during excimer laser light delivery in the femoral artery drastically reduced the incidence of dissections and internal elastic lamina ruptures. The saline in front of the catheter provided an optical window for direct irradiation of the arterial wall by the 308 nm excimer laser light, resulting in increased medial and adventitial necrosis. The direct irradiation of the arterial wall prevented, in part of the lesion,

restoration of the media and formation of intimal hyperplasia. At the edges of the irradiated lesions, however, intimal hyperplasia and arterial shrinkage decreased the arterial lumen with angiographic results that were equal to those after laser light delivery in a blood field.

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